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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

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Date of mailing:

16 December 1999 (16.12.99)

International application No.:

PCT/GB98/03727

Applicant's or agent's file reference:

PP/2394 PCT

International filing date:

03 December 1998 (03.12.98)

Priority date:

10 June 1998 (10.06.98)

Applicant:

THOMAS, Nicholas

1. The designated Office is hereby notified of its election made:



in the demand filed with the International preliminary Examining Authority on:

12 June 1999 (12.06.99)



in a notice effecting later election filed with the International Bureau on:

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Facsimile No.: (41-22) 740.14.35

Authorized officer:

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PP/2394 PCT	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 98/ 03727	International filing date (day/month/year) 03/12/1998	(Earliest) Priority Date (day/month/year) 04/12/1997
Applicant AMERSHAM PHARMACIA BIOTECH UK LIMITED; et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

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2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,



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the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.

1



None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/03727

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N33/58 C12Q1/00 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N C07B C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE WPI Section Ch, Week 9410 Derwent Publications Ltd., London, GB; Class A12, AN 94-077445 XP002102174 & JP 06 027112 A (NITTO DENKO CORP.) , 4 February 1994	1-10
X	see abstract	11
Y	WO 93 02360 A (TRANSMED BIOTECH INC) 4 February 1993	1-10
X	see claim 10	11
Y	WO 93 06121 A (AFFYMAX TECH NV) 1 April 1993	1-10
X	see figures 2,9	11
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

7 May 1999

Date of mailing of the international search report

19/05/1999

Name and mailing address of the ISA

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Griffith, G

II INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/03727

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 32425 A (SMITHKLINE BEECHAM CORP ;YAMASHITA DENNIS SHINJI (US); WEINSTOCK J) 30 November 1995	1-10
X	see page 22 - page 27 ---	11
Y	WO 97 14028 A (LUMINEX CORP ;CHANDLER VAN S (US); FULTON R JERROLD (US); CHANDLER) 17 April 1997	1-10
X	see page 7, line 1 - page 8, line 23 see figures 16A-C ---	11
Y	US 5 641 634 A (MANDECKI WLODEK) 24 June 1997 cited in the application	1-10
X	see example 3 ---	11
A	WO 95 13538 A (OPERON TECHNOLOGIES INC) 18 May 1995 see the whole document -----	1-11

INTERNATIONAL SEARCH REPORT

on patent family members

International Application No

PCT/GB 98/03727

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9302360	A	04-02-1993	AT 171543 T AU 2348092 A CA 2113350 A,C DE 69227112 D EP 0594763 A JP 6509417 T US 5567627 A	15-10-1998 23-02-1993 04-02-1993 29-10-1998 04-05-1994 20-10-1994 22-10-1996
WO 9306121	A	01-04-1993	AT 148889 T AU 669489 B AU 2661992 A CA 2118806 A DE 69217497 D DE 69217497 T DK 604552 T EP 0604552 A EP 0773227 A ES 2097925 T GR 3023156 T US 5639603 A US 5789162 A US 5708153 A US 5770358 A	15-02-1997 13-06-1996 27-04-1993 01-04-1993 27-03-1997 12-06-1997 04-08-1997 06-07-1994 14-05-1997 16-04-1997 30-07-1997 17-06-1997 04-08-1998 13-01-1998 23-06-1998
WO 9532425	A	30-11-1995	EP 0763202 A JP 10500951 T	19-03-1997 27-01-1998
WO 9714028	A	17-04-1997	US 5736330 A AU 7398996 A CA 2227895 A EP 0852004 A	07-04-1998 30-04-1997 17-04-1997 08-07-1998
US 5641634	A	24-06-1997	AU 1141597 A CA 2238696 A EP 0871777 A WO 9720074 A	19-06-1997 05-06-1997 21-10-1998 05-06-1997
WO 9513538	A	18-05-1995	NONE	



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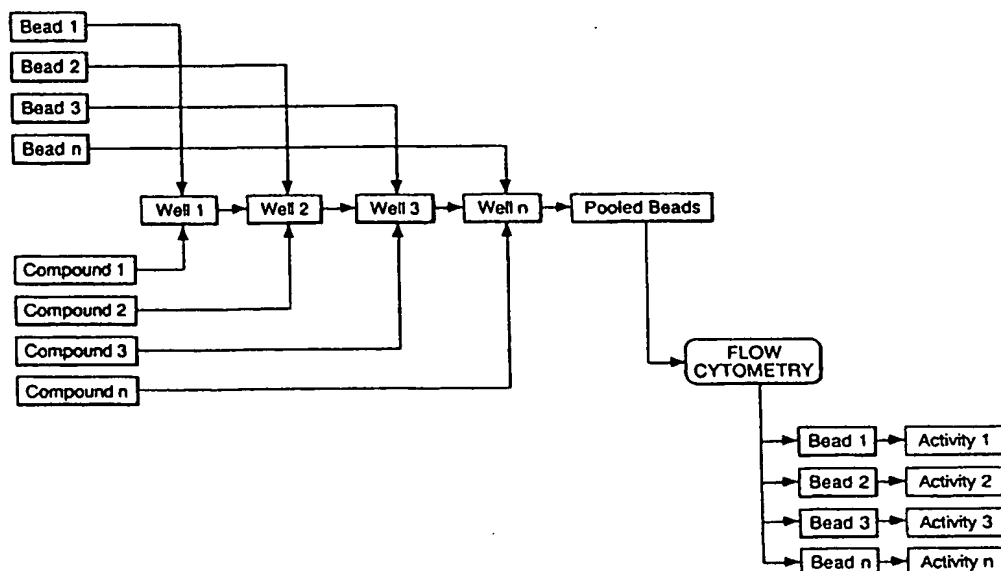
(74) Agents: PENNANT, Pyers et al.; Stevens Hewlett & Perkins, 1 Serjeants' Inn, Fleet Street, London EC4Y 1LL (GB).

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(54) Title: MULTIPLE ASSAY METHOD



(57) Abstract

A method for the assay of N samples each containing a compound to be tested, comprises providing N reaction vessels each containing a population of carrier beads and other reagents for performing the assay, where N is at least 2 e.g. 80–4000. Each population of carrier beads is distinguishable from every other population. After adding the samples to the reaction vessels and performing the assays, the contents of all the reaction vessels are mixed and subjected to analysis by flow cytometry. By means of flow cytometry, each carrier bead is rapidly analysed to identify its population and also to determine the presence or concentration or biological activity of the compound to be tested.

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MULTIPLE ASSAY METHOD

5 This invention relates to a multiple assay method suitable for performing high-throughput screening for drug discovery and specifically to a procedure which provides for large-scale parallel processing and analysis of results from many thousands of separate biological assays.

 The process of high-throughput screening (HTS) is central to
10 the objectives of the pharmaceutical industry, i.e. to discover, develop and market new drugs (Lutz *et al*, (1996) Drug Discovery Today, 1(7), 277-86). In the HTS process, drug candidates are screened for possible effects in biological systems. Increasingly, there is a drive to test larger numbers of compounds in each screen, and screening assays examining 100,000
15 compounds or more are typical. This requires highly sophisticated robotic automation and instrumentation to achieve efficient levels of throughput. In general, modern screening techniques utilise multiwell plate technologies to allow transfer of the many thousands of assays between the various stages in the procedure. Such plates may contain between 96 and 1536 or
20 more individual wells, where each well contains the same reagents as all other wells in the screen, except for the individual compounds under test which are each present in only one well. The standard format and layout of the multiwell plates allows fast robotic handling and liquid dispensing devices to be used to maximise throughput.

25 In many HTS applications the rate limiting step occurs in assay analysis at the stage of detecting and measuring the signal from the label used in the assay. This step is a serial process, each well of the multiwell plate being measured in turn. Such measurements typically require from one to several seconds to perform, with the consequence that
30 the time taken to analyse a multiwell plate can be considerable.

Flow Cytometry (Parks, D.R. and Herzenberg, L.A. 1984, Methods in Enzymology 108, 197-241) is a technique for analysing cells or particles according to their size and fluorescence. The cells or particles are carried by a thin rapidly moving stream of liquid which is transected by
5 light beam(s) from one or more lasers or other light sources. Photo-detectors register light-scattering and fluorescence arising from a cell or particle passing through a light beam and the resulting electronic signals are processed to yield analytical data. In contrast to the slow data acquisition time of multiwell plate readers, instrumentation for flow
10 cytometry enables very rapid analysis of many thousands or millions of cells or other particles in a high speed stream of liquid and is typified by very fast measurement times, for example of the order of 1 μ sec/event.

Flow cytometry has other characteristics which make it favourable for analysis in HTS. Firstly, the very small analysis volumes
15 required are compatible with the current trend to scale down assays as a means of increasing throughput. Secondly, flow cytometry is inherently an homogeneous measurement system, i.e. measurement of the fraction of a specific fluorescent dye-labelled ligand in a particular state can be accomplished without the need to physically separate that type of
20 fluorescent dye from the total type. In HTS applications, this is a desirable property as it removes the need for washing or separation stages to isolate the desired type of label prior to measurement. Flow cytometry has been extensively used for diagnostic assays to measure a wide range of analytes in blood and other biological fluids, for example in immunotyping
25 and measurement of cell surface antigens associated with HIV infection (Patterson, B.K. *et al* J. Virology, (1995) 69(7) 4316-4322). Despite its inherent advantages however, flow cytometry is disadvantaged by low throughput rates which are a consequence of serial processing. While read times are very fast, allowing many thousands of events to be
30 analysed/second within a single assay, there is a considerable delay

between samples which currently limits overall throughput to <100 separate analyses/hour.

A desire to have a higher throughput in these applications has led to the development of multiplex methods which allow more than one analyte to be measured simultaneously by flow cytometry. Multiplexing is achieved by carrying out solid phase linked assays using plastic or latex beads as assay substrates. By using a number of discrete bead types which are individually distinguishable from each other, where each bead type carries reagents for one assay, standard flow cytometer instrumentation may be used both to identify the bead type and to measure the assay signal associated with each bead, and therefore to perform several tests in parallel on a single sample, for example to measure the presence of multiple analytes in human sera (McHugh T.M., 1994, Methods in Cell Biology 42, 575-595). Discrimination between bead types can be achieved by size (Frengen J. *et al*, 1995, Journal of Immunological Methods, Volume 178, p141), by colour or fluorescence (Fulwyler M.J. UK Patent 1,561,042) or by electronic means (Mandecki W. US Patent 5,641,634).

Multiplexing of flow cytometry assays introduces an element of parallel processing into an otherwise serial process, so that while the delay between samples remains as before, the amount of information gathered from each sample is increased several fold giving a resulting increase in data acquisition rates. This is ideal for measurement of multiple analytes in a single sample, i.e. 'one sample, many tests'. However, the requirements of high throughput screening, i.e. 'one test, many samples', are the reverse. In HTS assays it is a requirement that there must always be separation of assays to allow the effects of individual compounds within the screen to be determined. Consequently, methods previously described for multiplex diagnostic analyses by flow cytometry are not applicable to HTS assays.

This invention provides a method for the assay of N samples each containing a compound to be tested, which method comprises the steps of:

- a) providing N populations of carrier beads where the carrier beads of each population are distinguishable from the carrier beads of every other population;
- b) dispensing each of the N populations of labelled carrier beads into one of N different reaction vessels;
- c) dispensing each of the N samples into one of the said different reaction vessels;
- d) providing in each of said N different reaction vessels reagents for performing an assay whereby a signal moiety is caused to be partitioned in a compound-related manner between the carrier beads in that reaction vessel and a supernatant fluid;
- e) combining the contents of all of the reaction vessels into a mixture; and
- f) subjecting the mixture to analysis by flow cytometry, to assay the signal moiety associated with each of a sequence of individual beads; wherein N is greater than or equal to 2.

The invention also provides a kit for performing the assay method, which kit comprises the N populations of the carrier beads where the carrier beads of each population are distinguishable from the carrier beads of every other population, and wherein all the beads are pre-coated with the same reagent for performing the assay, together with a supply of reagents for performing the assay, where N is at least 2.

Suitably, N is greater than or equal to 2; preferably in the range from 2 to 100,000, more preferably in the range from 80 to 4000.

Suitably, the carrier beads are coated with a reagent, bound thereto, the reagent optionally carrying a signal moiety.

Suitable assay formats which employ a reagent carrying

signal moiety bound to the carrier bead include those which, either by chemical or by enzymatic action, involve the release of a signal moiety from the bead. In the alternative, a reagent carrying the signal moiety is added in solution in a suitable medium, and include assays which involve
5 binding the signal moiety either covalently or non-covalently, to a reagent immobilised on the bead.

Suitably, the reaction vessels form the wells of a multiwell plate.

In step d) of the method, an assay reaction is performed in
10 which a signal moiety is caused to be partitioned in a compound related manner between carrier beads and a supernatant fluid. Various examples can be given. In one example, an assay is performed to determine the presence or absence in each sample of a particular compound to be tested; in each reaction vessel the signal moiety is partitioned in a manner
15 which indicates the presence or the absence of the compound in the sample dispensed in that vessel. In another example, an assay is performed to determine the concentration in each sample of a particular compound to be tested; in each reaction vessel the signal moiety is partitioned in a manner which indicates the concentration of the compound
20 in the sample dispensed in that vessel. In another example, which is preferred, an assay is performed to determine the biological activities of a plurality of different compounds to be tested, with each sample comprising or consisting of a different compound, generally in known amount; in each reaction vessel the signal moiety is partitioned in a manner which indicates
25 the biological activity of the compound in the sample dispensed in that reaction vessel.

Beads suitable for use in the method of the invention are those which are compatible with processing and analysis by flow cytometry and additionally are suitable for incorporating means of identification into
30 the bead. Preferred bead types are formed from plastic or polymeric

materials, including polystyrene latexes, polyacrylates, polymethylmethacrylate, polyacrylamides, polyurethane, polyvinylidene chloride and polyvinyltoluene. Polystyrene beads are particularly preferred for use in the invention. Beads may be of a mean diameter suitable for use
5 in flow cytometric applications. A bead size suitable for use in the invention may be in the range 1-50 μ m in diameter, preferably of diameter 2-20 μ m. Preferably, beads of the same size are used in the method of the invention. Optimally, beads of mean diameter 10 μ m are used in the method of this invention.

10 Detectable labels suitable for bead identification include fluorescent molecules, absorbed or incorporated into or onto the surface of the bead. As an alternative means of distinguishing and identifying bead populations, beads of one population may be of a different size compared with beads of another population. In a further alternative, bead
15 identification may be by electronic means, such as by the inclusion into the core of the bead of a suitable electronic tag. Detectable labels such as those described above may be used either singly or in combination to create bead populations which are uniquely identifiable.

Preferably beads including fluorescent labels are used in the
20 method of the invention. Fluorescent labelled beads suitable for use with the invention are prepared by the incorporation of different amounts of two or more different fluorescent dyes into the body of the bead such that each combination of such fluorescent dyes defines a unique bead type. The number of possible discrete assays that can be multiplexed will be limited
25 only by the number of bead types which it is possible to discriminate in a mixture. With current flow cytometry instrumentation, this does not pose a limitation on the utility of the procedure. Typical, modern flow cytometry instruments are capable of simultaneously measuring fluorescence at four wavelengths, together with other parameters, for example light scattering,
30 which is a measure of the size of particles under analysis. In addition, the

dynamic range of fluorescence detection is high and fluorescence may be accurately measured over several orders of magnitude. It is therefore possible to devise schemes which yield a large number of individually distinguishable bead types to serve as carriers in a HTS assay according to the present invention. For example, beads may be prepared which contain three separate, spectrally distinguishable fluorescent dyes, wherein each fluorescent dye may be present in one of 8 concentration levels. Thus it is possible to create 8^3 , that is 512 spectrally distinguishable bead types. If, in addition, 3 sizes of beads are used, the total number of bead types is $3 \times 512 = 1536$. This number is equivalent to the number of wells in a high density multiwell plate such as are suitable for use in HTS assays. Use of fluorescent dyes in such combinations will allow all reactions in a high density plate to be combined into a single sample for analysis by flow cytometry.

Suitable fluorescent dyes useful for bead identification are dyes which have discrete excitation and emission spectra suitable for individual identification in a flow cytometer. The exact chemical nature of the fluorescent dye is not critical to the present invention. Fluorescent dyes which may be used include, but are not limited to, fluoresceins, rhodamines, cyanine dyes, coumarins, and the BODIPY groups of fluorescent dyes. Methods for electronic coding and identification of beads are disclosed in US Patent 5,641,634.

The above described bead types can be applied to assays commonly used in HTS applications. Such assays are conveniently categorised as one of two types.

i) The first category comprises equilibrium binding assays, in which one member of a binding pair (the reactant) is bound to the surface of the bead and samples containing compounds to be screened are tested for their effect upon the binding, (either antagonistic or agonistic) to a second member of the binding pair (the ligand), where the

ligand carries a signal moiety, preferably a fluorescent label. In this way, the effect of the test sample on the binding reaction can be determined by measurement of the amount of labelled ligand bound to the bead through its interaction with its binding partner. Examples of such equilibrium
5 binding interactions include, but are not restricted to, receptor-ligand interactions, protein-protein binding interactions and protein-DNA interactions. Irrespective of the identity of the components of the interaction, all such assays are similarly characterised by having two components where one component is bound to the bead and the second
10 component carries a signal moiety which becomes attached to the bead through the interaction of the two components.

ii) In the second category, the assay may comprise detection and measurement of a chemical or enzymatic change in the state of an assay component bound to the bead and in which the samples
15 containing compounds to be screened are tested for their inhibitory effects, potentiation effects, agonistic, or antagonistic effects on the reaction under investigation. Illustrative of such reactions are those which include the removal of a fluorescent dye-labelled moiety from a substrate coupled directly or indirectly to the bead through a covalent or non-covalent
20 interaction, or alternatively, the covalent or non-covalent addition of a fluorescent dye-labelled moiety from a substrate in solution in the assay medium to a molecule coupled directly or indirectly to the bead by means of a covalent or non-covalent interaction. Examples of such assays include, but are not restricted to, the cleavage of a fluorescent dye-labelled
25 peptide or protein by a protease, the cleavage of fluorescent dye-labelled DNA or RNA molecule by a nuclease, the joining of fluorescent dye-labelled DNA or RNA molecules to other nucleic acid molecules by ligases, the addition of a fluorescent dye-labelled nucleotide to a DNA or RNA molecule by a polymerase and transfer of a fluorescent dye-labelled
30 chemical moiety from one molecule to another by a transferase such as

acetyl transferase.

Suitable fluorescent labels for tagging ligands or substrates are those which are: a) spectrally distinct and distinguishable from any fluorescent dye used for bead identification, b) detectable by a flow
5 cytometer and, c) capable of being attached to the ligand or substrate component of the assay by covalent or non-covalent attachment. Suitable fluorescent dyes for use in labelling ligands and substrates according to the method of the invention may be selected from the general categories of fluorescent dyes listed above. Preferably, derivatives of such fluorescent
10 dyes having reactive or functional groups suitable for attachment to corresponding functional or reactive groups on biological target molecules are used. Examples of such reactive fluorescent dyes are sulpho-cyanine dye NHS ester derivatives as described in US Patent No.4268486 (Waggoner *et al*). Other fluorescent reagents suitable for labelling target
15 molecules will be well known to those skilled in the art.

Alternatively, labels useful for attachment to the ligands or substrates can be fluorescence energy transfer labels. Examples of such energy transfer fluorescent dyes are to be found in GB Patent No.2301833 (Waggoner *et al*) which relates to fluorescent energy transfer complexes
20 containing reactive or functional groups for covalent attachment to a target. Other fluorescent energy transfer labels may be bound non-covalently to a ligand or substrate moiety, e.g. by intercalation of a dye to a dsDNA molecule. Examples of such dyes are disclosed in US Patent No.5401847 (Glazer *et al*).

25 For clarity, the general principles and specific embodiments of the method according to the present invention (termed mix/multiplex HTS), are described with reference to the following figures:

Figure 1: Flowchart illustrating the principle of the mix/multiplex HTS process.

30 **Figure 2:** Schematic representation of the mix/multiplex HTS

process using fluorescence bead identification.

Figure 3: Schematic representation of the mix/multiplex HTS process using electronic bead identification.

With reference to Figure 1, to perform the mix/multiplex HTS process, sufficient individual types of carrier beads are used to allow one carrier bead type for each discrete assay to be performed in a screening unit, that is one bead type for each sample to be screened. The surfaces of beads are modified, for example by coating with a binding reagent such as an antibody, protein A, streptavidin, avidin, wheat germ agglutinin or poly-l-lysine. Alternatively the bead surface may be treated by chemical modification to provide functional or reactive groups suitable for the attachment of specific assay components such as proteins, peptides, oligonucleotides, ligands and carbohydrates to the surface of the bead. Suitable functional or reactive groups include hydroxyl, amino, carbonyl, carboxyl and sulphydryl groups. Methods suitable for coupling reactants to the surface the bead are well known to those skilled in the art.

In a further illustration of the method of this invention, reference is made to two possible but non-restrictive embodiments of the process. With reference to Figure 2, beads containing different amounts of two fluorescent dyes are used as carrier beads for the assay. To set up the assay, each type of beads (2,3,4) is added to a separate well of a multiwell screening plate (1). Pre-dispensed plates arrayed in this manner are then used as the basis for the screening process as described generally above. Once the reaction stage of the screening process is complete, a portion, or the entire contents of each of the wells in which reactions have been conducted, are mixed together into a single container (6) and analysed by flow cytometry. In the flow cytometer, two of the available fluorescence measurement channels are used to identify the bead type to which any individual bead belongs, by determining the amounts of the two fluorescent dyes present within the bead.

Simultaneously, a third fluorescence channel may be used to quantify the amount of fluorescent dye-labelled ligand or substrate bound to the surface of the bead.

In performing assays according to the method of this invention, it is convenient to refer to a screening unit which will typically correspond to assay reactions performed in one multiwell plate, where a complete screen comprises from one to many multiwell plates. Each bead type from 1-N is individually added to corresponding wells 1-N containing assay reagents, where for example in the case of a receptor based screening assay, reagents would typically comprise a receptor preparation, assay buffer and a fluorescent labelled receptor ligand. Samples containing one or more compounds to be screened are added individually to the prepared wells, samples 1-N being added to wells 1-N. In this way, a fixed correspondence is established between each sample in the screen and the bead type carrying the reactants exposed to each individual sample containing one or more compounds under test. Once the reaction stage of the assay is complete, all reactions in a screening unit are mixed together for analysis without destroying the correspondence, since on analysis by flow cytometry, both the particular bead type and the assay signal associated with it can be readily determined for any given bead in the analysed mixture. Accordingly, once analysis of the mixed samples is complete, assay signals measured from beads 1-N (activity 1-N) can be correlated with samples 1-N originally added to wells 1-N.

Display of data as an x-y-z plot (7) allows identification of individual bead types according to the relative intensities of fluorescence from bead fluorescent dyes 1 (8) and 2 (9), on the x and y axes respectively and allows intensities of assay signals for each compound in the screen to be displayed separately (10). The identity of each bead type can be determined from its x-y position and therefore the z assay signal of that type can be assigned to a single well in the original multiwell plate.

Consequently all assay data from one mixed sample can be displayed as a data matrix (11) corresponding to the original layout of the multiwell plate and results examined to determine the activity of the compounds screened.

In a second possible embodiment of the process of the invention, electronic encoding is used to identify assay carrier beads. With reference to Figure 3, in this embodiment carrier beads containing semiconductor memory devices, as described in US Patent 5,641,634, and coated with assay reagents particular to the type of assay being performed are used in a bulk suspension (12) as described above. The bulk beads are dispensed into multiwell plates (13) using a dispensing nozzle (14) fitted with a radio-frequency generating coil (16) controlled by encoding circuitry (15). This apparatus allows beads passing through the dispensing device to be given a unique identity through action of the radio-frequency field on the semiconductor. By this means it is possible, starting with a bulk suspension of identical beads and by moving the dispensing device from well to well, to produce a multiwell plate with an uniquely coded population of beads in each well. At this stage the screening process is continued as described above with assay reagents (17) being added to the wells of the multiwell plate, followed by samples (18) containing compounds to be screened.

For analysis, a modified flow cytometer (20) is used where the instrument is fitted with a second radio-frequency coil (22) set up to read information encoded on the semiconductor within beads through decoding circuitry (21). Pooled beads (19) passing through the instrument are first read by the decoder and secondly by the instrument's conventional fluorescence detection laser (23) and photomultiplier (24) components to give a continuous data readout of bead identity code (25) and assay fluorescence signal (26).

By the use of electronic encoding as a means of bead identification, the possible number of bead types is limited only by the

capacity of the semiconductor. For example a 16 bit device would allow the characterisation of 32768 different bead types and therefore mix/multiplexing of 32768 discrete screening assay reactions. Secondly, beads may be prepared for an assay in bulk and coded directly before use, thereby removing the need to carry out preparations on each bead type individually. Radio-frequency encoding and reading removes the requirement for multiple fluorescence channels to be used for bead identification allowing either simplification of instrumentation or use of fluorescence channels to measure additional assay information.

10 The mix/multiplex procedure as described above allows very high throughput of individual screening assay reactions by analysis by flow cytometry in a manner which exploits the capabilities of flow cytometry instrumentation, and in particular the very fast data acquisition which can be obtained. The procedure is compatible with the wide range of different sized multiwell plates that are commonly used in HTS programs. The method of this invention is preferably used in high well density, small well volume, plates such as 1536 well plates, where the assay volume/well is 10 μ l or less. The amounts of beads used in each well may be varied to accommodate the requirements of different screening assays, but will preferably be in the range 0.01-10%v/v in respect of the assay volume, most preferably in the range 0.1-1.0%v/v. At the most preferred bead concentrations and using a preferred bead size of 10 μ m, a single assay well containing 10 μ l of liquid would contain a number of beads in the range 10,000-100,000 beads/well.

25 Each bead in any individual well is identical to every other bead in the same well and is therefore an individual assay unit which can be separately measured by flow cytometry. In performing biological assays, it is common practice to perform replicate measurements in order to take account of physical or biological variations inherent to the assay process. Such replicates typically take the form of duplicate or triplicate

30

determinations of each assay which are carried out to obtain data typically expressed as a mean \pm standard deviation, where the standard deviation is a statistical measure of the variation in the assay data which is used to assess the precision and accuracy of the data obtained. In conventional screening assays the assay comprises the whole well or tube in which the assay is performed, and therefore replication involves duplication of the entire assay. In contrast in bead based assays where each bead is an measurable unit, replicate measurements may be performed at the level of individual beads. Therefore, while an assay well may contain 10,000-100,000 beads it is not necessary in the subsequent analysis to measure every bead from that well, but only to measure sufficient beads to accumulate data which meets predetermined specifications for precision and accuracy.

The potential throughput of the process is illustrated by the following example. If a 1536 well plate is used as single screening unit, and it is determined that to obtain a statistically valid analysis it is necessary to measure assay results for 100 beads for each compound screened, the total number of beads to be analysed is 153,600. Modern flow cytometers are readily capable of performing measurements on between 1000 and 10,000 particles/second; assuming analysis at an intermediate rate of 2500 beads/second yields an analysis time of $153,600/2500 = 61.44$ seconds or approximately 1 minute to measure the 1536 discrete assays in the multiwell plate. This compares very favourably with a time of 25.6 minutes to read results individually in a plate reader at a speed of 1 second/well. Allowing for loading of successive mixed samples on to a flow cytometer at a rate of 40/hour gives a throughput of $1536 \times 40 = 61440$ assays/hour or around 500,000 assays in a working day.

Example

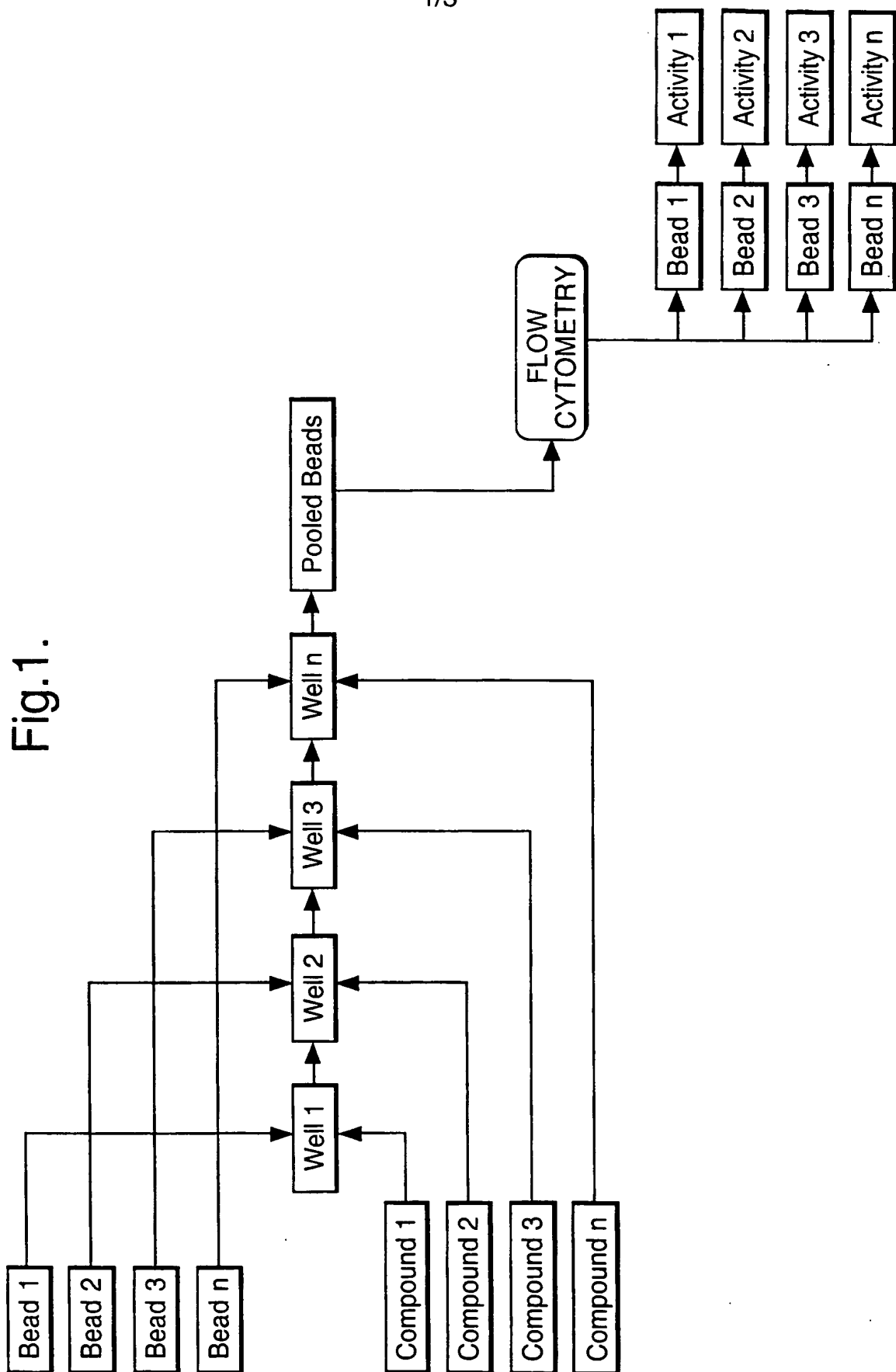
Streptavidin coated bead type A (yellow fluor) and bead type B (purple fluor) were pipetted into two separate wells of a microtitre plate, such that well 1 contained bead A and well 2 contained bead B. Buffer was added to well 1 and buffer containing biotin was added to well 2. Following mixing and incubation a solution of Cy-5 labelled biotin in buffer was added to both wells. Following further incubation the contents of well 1 and well 2 were combined and the mixture analysed by flow cytometry. Measurement of Cy-5 fluorescence associated with each bead type showed high fluorescence for bead B indicating high Cy-5-biotin binding in well 1 and low Cy-5-biotin binding in well 2, correlating with the presence in well 2 of an active competitor for binding to the streptavidin on the bead surface.

CLAIMS

- 5 1. A method for the assay of N samples each containing a compound to be tested, which method comprises the steps of:
- a) providing N populations of carrier beads where the carrier beads of each population are distinguishable from the carrier beads of every other population;
 - 10 b) dispensing each of the N populations of labelled carrier beads into one of N different reaction vessels;
 - c) dispensing each of the N samples into one of the said different reaction vessels;
 - d) providing in each of said N different reaction vessels reagents
 - 15 for performing an assay whereby a signal moiety is caused to be partitioned in a compound-related manner between the carrier beads in that reaction vessel and a supernatant fluid;
 - e) combining the contents of all of the reaction vessels into a mixture; and
 - 20 f) subjecting the mixture to analysis by flow cytometry, to assay the signal moiety associated with each of a sequence of individual beads; wherein N is greater than or equal to 2.
2. A method as claimed in claim 1, wherein in step a) there are
- 25 provided N populations of carrier beads where the carrier beads of one population are distinguishable by virtue of a detectable label from the carrier beads of another population.
3. A method as claimed in claim 1 or claim 2, wherein N is
- 30 80 - 100,000.

4. A method as claimed in claim 2 and claim 3, wherein in step f) the mixture is subjected to analysis by flow cytometry, to assay the signal moiety and the label associated with each of a sequence of individual beads, whereby the signal moiety indicates the biological activity of the compound to be tested and the label indicates the sample containing the compound.
5. A method as claimed in any one of claims 1 to 4, wherein N is from 80 to 4000.
6. A method as claimed in any one of claims 1 to 4, wherein the beads are pre-coated with a reagent for performing the assay.
7. A method as claimed in any one of claims 1 to 6, wherein a population of beads is detectably labelled by means of at least one fluorescent dye.
8. A method as claimed in any one of claims 1 to 6, wherein a population of beads is electronically labelled.
9. A method as claimed in any one of claims 1 to 8, wherein the signal moiety is a fluorescent dye.
10. A method as claimed in any one of claims 1 to 9, wherein in step d) the same reagents for performing the same assay are provided in each of the N different reaction vessels.

11. A kit for the assay of N samples each containing one or more compounds to be tested, which kit comprises N populations of carrier beads where the carrier beads of each population are distinguishable from the carrier beads of every other population, and wherein all the beads are
5 pre-coated with the same reagent for performing the assay, together with a supply of reagents for performing the assay, where N is at least 2.



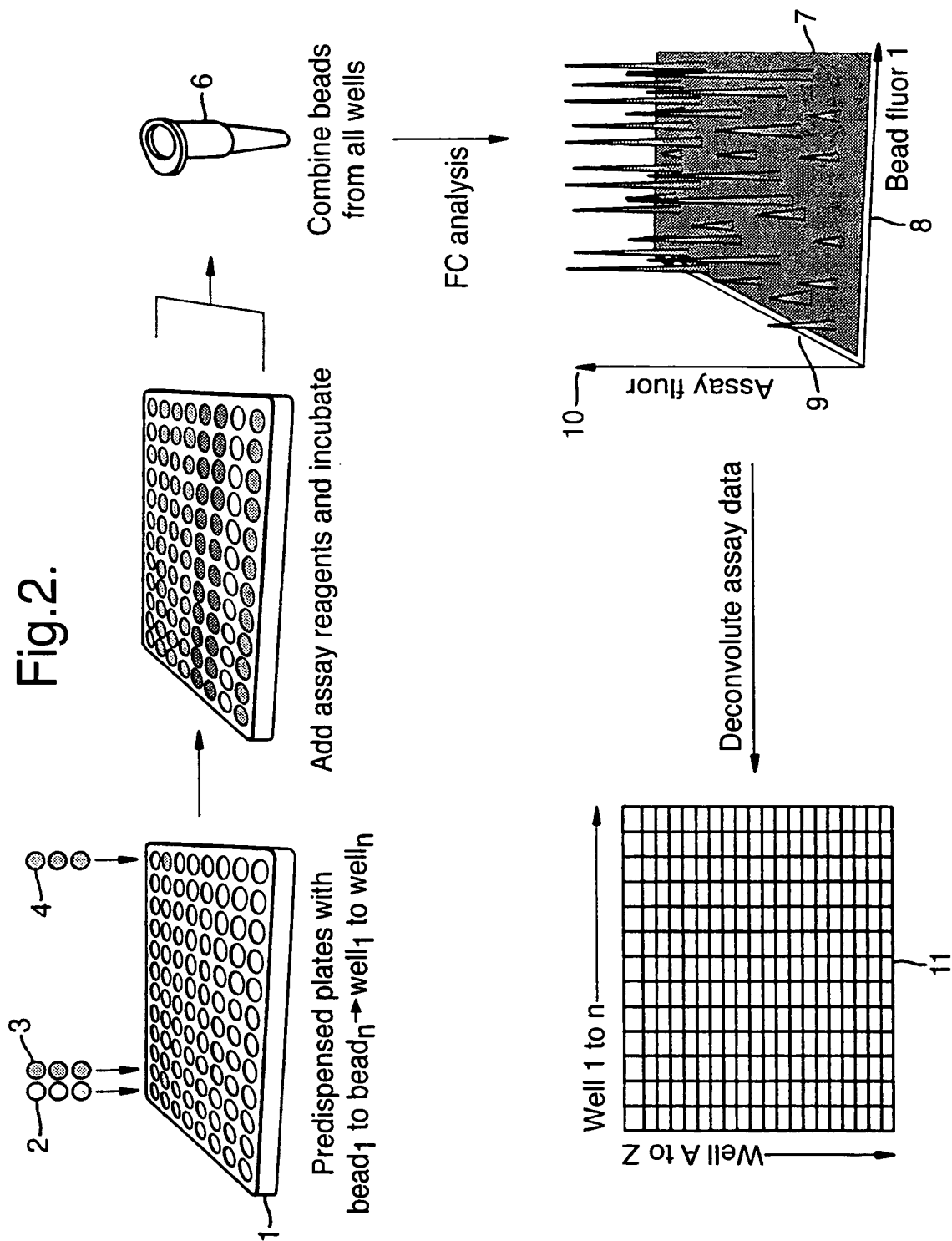
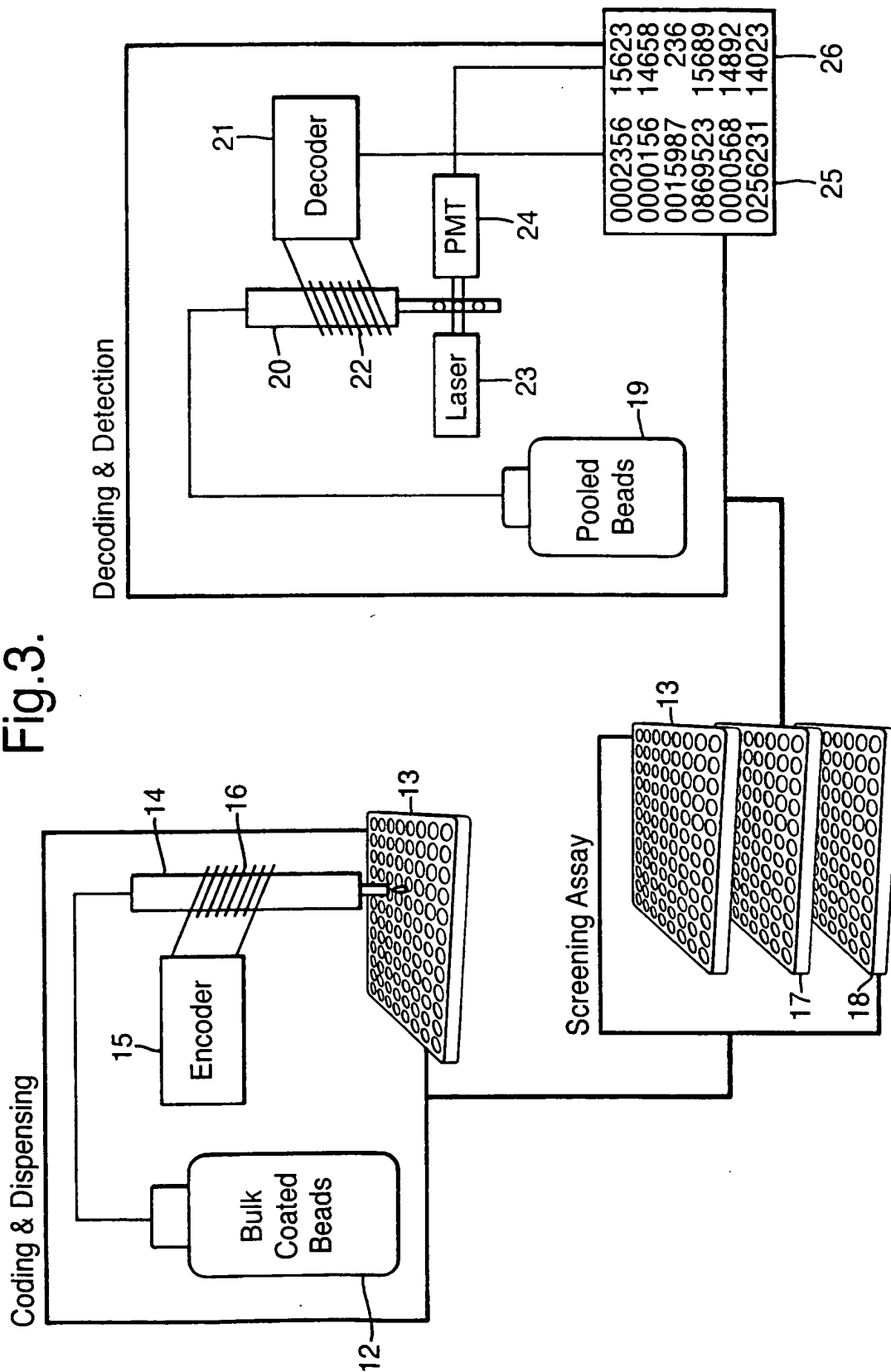


Fig.3.





09/555102 PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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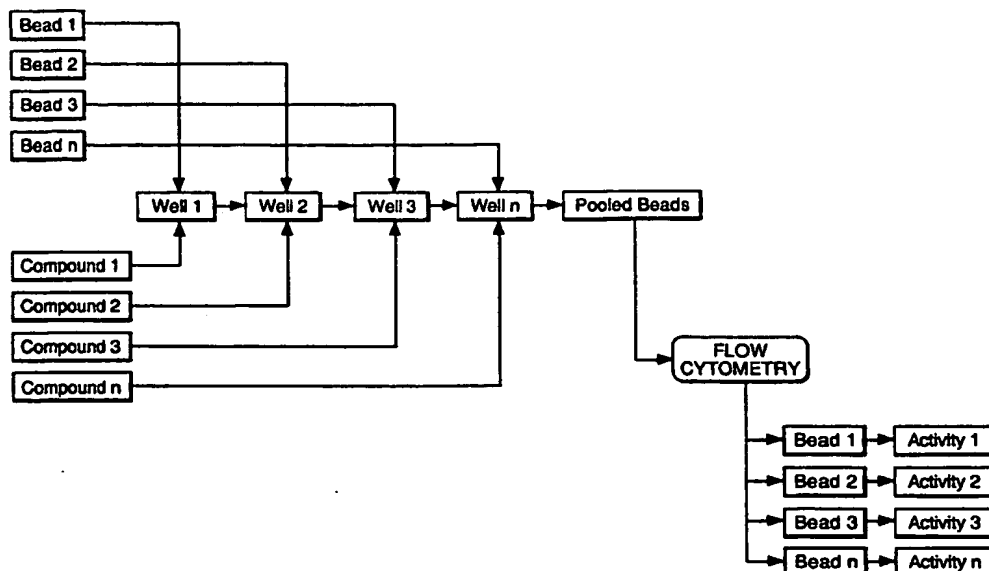
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(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

With international search report.

(54) Title: MULTIPLE ASSAY METHOD



(57) Abstract

A method for the assay of N samples each containing a compound to be tested, comprises providing N reaction vessels each containing a population of carrier beads and other reagents for performing the assay, where N is at least 2 e.g. 80-4000. Each population of carrier beads is distinguishable from every other population. After adding the samples to the reaction vessels and performing the assays, the contents of all the reaction vessels are mixed and subjected to analysis by flow cytometry. By means of flow cytometry, each carrier bead is rapidly analysed to identify its population and also to determine the presence or concentration or biological activity of the compound to be tested.

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PP/2394 PCT		FOR FURTHER ACTION		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB98/03727	International filing date (day/month/year) 03/12/1998	Priority date (day/month/year) 10/06/1998		
International Patent Classification (IPC) or national classification and IPC G01N33/58				
Applicant AMERSHAM PHARMACIA BIOTECH UK LIMITED et al.				

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 12/06/1999	Date of completion of this report 28.03.2000
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Maucher, C Telephone No. +49 89 2399 7415 

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB98/03727

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-3,5-15	as originally filed		
4,4a	as received on	09/03/2000 with letter of	07/03/2000

Claims, No.:

1-10	as originally filed		
11	as received on	09/03/2000 with letter of	07/03/2000

Drawings, sheets:

1/3-3/3	as originally filed
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2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB98/03727

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB98/03727

Point V:

Reference is made to the following documents:

D1 : WO-A-93 02360

D2: WO-A-97 14028

D1 discloses a method and kit (page 21, last full paragraph, claim 34) for contiguously detecting multiple analytes (360 contiguous assays; page 7, line 8) of interest in a sample comprising combining a sample with a composition comprising known proportions of multiple discrete subpopulations of reagents, which bind specifically to analytes, which are linked to particulate supports (e.g. microspheres; claim 17) and which may be detected by flow cytometry. The specific binding pairs are contacted with an agent labelled with a fluorochrome. A particular fluorescence intensity is obtained for each discrete population of specific binding pairs (claim 10).

D2 describes a method and kit (claim 82) for the multiplexed diagnostic and genetic analysis of enzymes, DNA fragments, antibodies etc. (abstract). The invention employs a pool of bead subsets, the beads of one subset differing in at least one distinguishing characteristic from beads in any other subset (page 7, 1. paragraph). Prior to pooling, different subsets will be coupled to different reactants so as to detect different analytes (page 7, 3. paragraph). Different subsets of beads may also be coupled to the same reactant (page 8, line 26). The variously labelled (e.g. with 2 or more fluorochromes; page 8, line 15) subsets of pooled beads are mixed with a fluid sample to test for different analytes reactive with the reactants bound to the beads (page 7, line 17 ff.). For example, 2 different subsets are used (page 7, lines 18-19). This mixture is examined in a flow cytometer (page 8, 1. line).

1. Article 33(2) PCT

- 1.1. The subject-matter of claims 1-10 is novel (Article 33(2) PCT) in the light of the closest prior art D1 or D2. It is distinguished from the prior art in that several samples containing different analytes are examined at the same time.

1.2. Claim 11 is novel (Article 33(2) PCT) in the light of D2 (see summary above), since claim 11 discloses a kit comprising different populations of beads which are all precoated with the same reagent "at substantially the same surface concentration".

2. Article 33(3) PCT

2.1. The subject-matter of claim 1 is inventive (Article 33(3) PCT), since none of the available prior art documents discloses an improved (faster) method for assaying different samples as disclosed in the present application. The solution to mix all the contents of all the reaction vessels containing the different samples with different analytes and to perform flow cytometry on said mix could not be derived from any prior art document, either if taken alone or in any combination.

The same applies to dependent claims 2-10.

2.2. Claim 11 is inventive (Article 33(3) PCT), since no prior art document discloses the use of different populations of beads with substantially the same reagent at the same surface concentration, either if taken alone or in any combination. This makes simultaneous examination of different reaction conditions possible for one particular compound to be tested, i.e. mixing of different reactions, due to the distinguishable bead populations (each population marking another reaction) (see the example of page 15).

Point VIII:

Claim 11 does not meet the requirements of Article 6 PCT. The IPEA does not see how different compounds in one sample can be distinguished or how the one compound being responsible for a certain effect can be identified among other compounds in the same sample when using beads precoated with the same reagent.

This invention provides a method for the assay of N samples each containing a compound to be tested, which method comprises the steps of:

- a) providing N populations of carrier beads where the carrier beads of each population are distinguishable from the carrier beads of every other population;
- b) dispensing each of the N populations of labelled carrier beads into one of N different reaction vessels;
- c) dispensing each of the N samples into one of the said different reaction vessels;
- d) providing in each of said N different reaction vessels reagents for performing an assay whereby a signal moiety is caused to be partitioned in a compound-related manner between the carrier beads in that reaction vessel and a supernatant fluid;
- e) combining the contents of all of the reaction vessels into a mixture; and
- f) subjecting the mixture to analysis by flow cytometry, to assay the signal moiety associated with each of a sequence of individual beads; wherein N is greater than or equal to 2.

The invention also provides a kit for performing the assay method, which kit comprises the N populations of the carrier beads where the carrier beads of each population are distinguishable from the carrier beads of every other population, and wherein all the beads are pre-coated with the same reagent for performing the assay, together with a supply of reagents for performing the assay, where N is at least 2.

Suitably, N is greater than or equal to 2; preferably in the range from 2 to 100,000, more preferably in the range from 80 to 4000.

Suitably, the carrier beads are coated with a reagent, bound thereto, the reagent optionally carrying a signal moiety.

Suitable assay formats which employ a reagent carrying

11. A kit for the assay of N samples each containing one or more compounds to be tested, which kit comprises N populations of carrier beads where the carrier beads of each population are distinguishable from the carrier beads of every other population, and wherein all the beads are
- 5 pre-coated with the same reagent for performing the assay, together with a supply of reagents for performing the assay, where N is at least 2.

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY: HEWLETT

PCT

WRITTEN OPINION

(PCT Rule 66)

To:

PENNANT, Pyers
STEVENS HEWLETT & PERKINS
1 Serjeant's Inn
Fleet Street
London EC4Y 1NT
GRANDE BRETAGNE

24 JAN 2000

29.2.

14.2.

Date of mailing:
(day/month/year)

14. 12. 99

Applicant's or agent's file reference

PP/2394 PCT

OF

REPLY DUE

within 3 month(s)
from the above date of mailing

International application No.

PCT/GB98/03727

International filing date (day/month/year)

03/12/1998

Priority date (day/month/year)

10/06/1998

International Patent Classification (IPC) or both national classification and IPC

G01N33/58

Applicant

AMERSHAM PHARMACIA BIOTECH UK LIMITED et al.

1. This written opinion is the first drawn up by this International Preliminary Examining Authority.
2. This opinion contains indications relating to the following items:
 - I ☒ Basis of the opinion
 - II ☐ Priority
 - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☐ Lack of unity of invention
 - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI ☐ Certain document cited
 - VII ☒ Certain defects in the international application
 - VIII ☒ Certain observations on the international application
3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.
4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 10/10/2000.

Name and mailing address of the international preliminary examining authority:



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner

Maucher, C

Formalities officer (incl. extension of time limits)

Danti, B

Telephone No. +49 89 2399 8161



WRITTEN OPINION

International application No. PCT/GB98/03727

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-15 as originally filed

Claims, No.:

1-11 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	11
Inventive step (IS)	Claims	11
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Point V:

Reference is made to the following documents:

D1 : WO-A-93 02360

D2: WO-A-97 14028

D1 discloses a method and kit (page 21, last full paragraph, claim 34) for contiguously detecting multiple analytes (360 contiguous assays; page 7, line 8) of interest in a sample comprising combining a sample with a composition comprising known proportions of multiple discrete subpopulations of reagents, which bind specifically to analytes, which are linked to particulate supports (e.g. microspheres; claim 17) and which may be detected by flow cytometry. The specific binding pairs are contacted with an agent labelled with a fluorochrome. A particular fluorescence intensity is obtained for each discrete population of specific binding pairs (claim 10).

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1. Article 33(2) PCT

- 1.1. The subject-matter of claims 1-10 appears to be novel (Article 33(2) PCT) in the light of the closest prior art D1 or D2. It is distinguished from the prior art in that

several samples containing different analytes are examined at the same time.

- 1.2. Claim 11 is not considered as being novel (Article 33(2) PCT), since the subject-matter of said claim seems to be already disclosed in D1 and D2 (see summaries above).

If claim 11 is to be interpreted as comprising different populations of beads which are all precoated with the same reagent for determining one single analyte, then claim 11 is novel (Article 33(2) PCT) in the light of D1 (see summary above).

2. Article 33(3) PCT

- 2.1. The subject-matter of claim 1 appears to be inventive (Article 33(3) PCT), since none of the available prior art documents discloses an improved (faster) method for assaying different samples as disclosed in the present application. The solution to mix all the contents of all the reaction vessels containing the different samples with different analytes and to perform flow cytometry on said mix could not be derived from any prior art document, either if taken alone or in any combination.

The same applies to dependent claims 2-10.

- 2.2. Concerning claim 11, should the objection as to novelty be overcome (see 1.2.), the following is to be said about inventiveness (Article 33(3) PCT). D2 discloses a similar method to the one disclosed in D1 with the embodiment to couple the same reactant to different subsets of beads (see summary above). Thus, in view of D1 and D2, claim 11 is not considered inventive (Article 33(3) PCT).

Point VII:

1. To meet the requirements of Rule 5.1(a)(ii) PCT, the documents D1-D2 should be identified in the description and the relevant background art disclosed therein should be briefly discussed.

2. The applicant should take care during revision, especially of the introductory portion and any statements of problem or advantage, not to add subject-matter which extends beyond the content of the application as originally filed (Articles 19(2) and 34(2)(b) PCT).
3. In order to facilitate the examination of the conformity of the amended application with the requirements of Articles 19(2) and 34(2)(b) PCT, the applicant is requested to clearly identify the amendments carried out, irrespective of whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based.

These indications should be submitted in handwritten form on a copy of the relevant parts of the application as filed.

Point VIII:

Claim 1.1 appears to be inconsistent (Article 6 PCT), because if there are different compounds to be tested, the carrier beads can't be precoated with the same reagent. For the present examination both possibilities have been taken into account, i.e. only one compound and one reagent as well as more compounds and more reagents.

PATENT COOPERATION TREATY

PCT

REC'D 30 MAR 2000

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

PCT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PP/2394 PCT	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB98/03727	International filing date (day/month/year) 03/12/1998	Priority date (day/month/year) 10/06/1998
International Patent Classification (IPC) or national classification and IPC G01N33/58		
Applicant AMERSHAM PHARMACIA BIOTECH UK LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.


2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 12/06/1999	Date of completion of this report 28.03.2000
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Maucher, C Telephone No. +49 89 2399 7415



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB98/03727

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-3,5-15	as originally filed			
4,4a	as received on	09/03/2000	with letter of	07/03/2000

Claims, No.:

1-10	as originally filed			
11	as received on	09/03/2000	with letter of	07/03/2000

Drawings, sheets:

1/3-3/3	as originally filed
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2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB98/03727

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB98/03727

Point V:

Reference is made to the following documents:

D1 : WO-A-93 02360

D2: WO-A-97 14028

D1 discloses a method and kit (page 21, last full paragraph, claim 34) for contiguously detecting multiple analytes (360 contiguous assays; page 7, line 8) of interest in a sample comprising combining a sample with a composition comprising known proportions of multiple discrete subpopulations of reagents, which bind specifically to analytes, which are linked to particulate supports (e.g. microspheres; claim 17) and which may be detected by flow cytometry. The specific binding pairs are contacted with an agent labelled with a fluorochrome. A particular fluorescence intensity is obtained for each discrete population of specific binding pairs (claim 10).

D2 describes a method and kit (claim 82) for the multiplexed diagnostic and genetic analysis of enzymes, DNA fragments, antibodies etc. (abstract). The invention employs a pool of bead subsets, the beads of one subset differing in at least one distinguishing characteristic from beads in any other subset (page 7, 1. paragraph). Prior to pooling, different subsets will be coupled to different reactants so as to detect different analytes (page 7, 3. paragraph). Different subsets of beads may also be coupled to the same reactant (page 8, line 26). The variously labelled (e.g. with 2 or more fluorochromes; page 8, line 15) subsets of pooled beads are mixed with a fluid sample to test for different analytes reactive with the reactants bound to the beads (page 7, line 17 ff.). For example, 2 different subsets are used (page 7, lines 18-19). This mixture is examined in a flow cytometer (page 8, 1. line).

1. Article 33(2) PCT

- 1.1. The subject-matter of claims 1-10 is novel (Article 33(2) PCT) in the light of the closest prior art D1 or D2. It is distinguished from the prior art in that several samples containing different analytes are examined at the same time.

- 1.2. Claim 11 is novel (Article 33(2) PCT) in the light of D2 (see summary above), since claim 11 discloses a kit comprising different populations of beads which are all precoated with the same reagent "at substantially the same surface concentration".

2. Article 33(3) PCT

- 2.1. The subject-matter of claim 1 is inventive (Article 33(3) PCT), since none of the available prior art documents discloses an improved (faster) method for assaying different samples as disclosed in the present application. The solution to mix all the contents of all the reaction vessels containing the different samples with different analytes and to perform flow cytometry on said mix could not be derived from any prior art document, either if taken alone or in any combination.

The same applies to dependent claims 2-10.

- 2.2. Claim 11 is inventive (Article 33(3) PCT), since no prior art document discloses the use of different populations of beads with substantially the same reagent at the same surface concentration, either if taken alone or in any combination. This makes simultaneous examination of different reaction conditions possible for one particular compound to be tested, i.e. mixing of different reactions, due to the distinguishable bead populations (each population marking another reaction) (see the example of page 15).

Point VIII:

Claim 11 does not meet the requirements of Article 6 PCT. The IPEA does not see how different compounds in one sample can be distinguished or how the one compound being responsible for a certain effect can be identified among other compounds in the same sample when using beads precoated with the same reagent.

WO-A-93/02360 discloses a method and kit for contiguously detecting multiple analytes of interest in a sample comprising combining a sample with a composition comprising known proportions of multiple discrete sub populations of reagents, which bind specifically to analytes, which are linked to particulate supports e.g. microspheres and which may be detected by flow cytometry.

WO-A-97/14028 describes a method and kit for the multiplexed diagnostic and genetic analysis of enzymes, DNA fragments, antibodies etc. The invention employs a pool of bead subsets, the beads of one subset differing in at least one distinguishing characteristic from beads of any other subset.

This invention provides a method for the assay of N samples each containing a compound to be tested, which method comprises the steps of:

- a) providing N populations of carrier beads where the carrier beads of each population are distinguishable from the carrier beads of every other population;
- b) dispensing each of the N populations of labelled carrier beads into one of N different reaction vessels;
- c) dispensing each of the N samples into one of the said different reaction vessels;
- d) providing in each of said N different reaction vessels reagents for performing an assay whereby a signal moiety is caused to be partitioned in a compound-related manner between the carrier beads in that reaction vessel and a supernatant fluid;
- e) combining the contents of all of the reaction vessels into a mixture; and
- f) subjecting the mixture to analysis by flow cytometry, to assay the signal moiety associated with each of a sequence of individual beads; wherein N is greater than or equal to 2.

The invention also provides a kit for performing the assay method, which kit comprises the N populations of the carrier beads where the carrier beads of each population are distinguishable from the carrier beads of every other population, and wherein all the beads are pre-coated
5 with the same reagent at substantially the same surface concentration for performing the assay, together with a supply of reagents for performing the assay, where N is at least 2.

Suitably, N is greater than or equal to 2; preferably in the range from 2 to 100,000, more preferably in the range from 80 to 4000.

10 Suitably, the carrier beads are coated with a reagent, bound thereto, the reagent optionally carrying a signal moiety.

Suitable assay formats which employ a reagent carrying

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- 18 -

11. A kit for the assay of N samples each containing one or more compounds to be tested, which kit comprises N populations of carrier beads where the carrier beads of each population are distinguishable from the carrier beads of every other population, and wherein all the beads are
- 5 pre-coated with the same reagent at substantially the same surface concentration for performing the assay, together with a supply of reagents for performing the assay, where N is at least 2.